0022-202X/78/7103-0172$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 71:172–176, 1978

Vol. 71, No. 3
Printed in U.S.A.

REPORTS

Inhibition of Connective Tissue Proliferation by Dermal Extract

CARL T. HANKS, D.D.S., PH.D., AND ELLEN O. SMITH, PH.D.

Department of Oral Pathology and Dental Research Institute, University of Michigan, Ann Arbor, Michigan, U.S.A

Following the subcutaneous injection of a water soluble dermal extract (DE) of neonatal rat skin into young adult male rats, depression of nuclear labeling (DNA synthesis) was observed in proliferating connective tissue in several wound sites. At 16–20 hr following DE injection, DNA synthesis was depressed most in back wounds (57–87%) and maxillary palatal wounds (45–68%), and least in ear wounds (24–29%). Epithelium in the wound margins of back, ear and palate did not show a similar depression in DE-injected animals. This study suggests that a chalone-like negative feedback mechanism may be partially responsible for *in vivo* control of fibroblastic proliferation in wound healing.

In cutaneous and mucosal excisional wounds, several processes such as vasodilatation, inflammatory cell infiltration and replacement of injured tissues, are taking place simultaneously. Granulation tissue plays a major role in repairing connective tissue [1] as well as permitting epithelial migration from the wound margin [2]. Connective tissue proliferation may occur as far as 1 mm away from the zones of tissue necrosis and inflammatory cell infiltrate in the region of the clot [3,4]. This suggests that the process is probably derived from sources other than tissue destruction, e.g., serum factors [5–8].

Although the theoretical concept of negative feedback control over tissue and organ growth was presented as early as 1957 [9], it was not until 1972 that a specific endogenous inhibitor of fibroblastic DNA synthesis was demonstrated in vitro [10]. The objective of the present study was to determine if a crude dermal extract, prepared in a manner similar to epidermal chalones, could alter DNA synthesis in wounded connective tissue.

METHODS

Wounds and Granulation Tissue

Young, male Sprague-Dawley rats, each weighing approximately 175 gm were injured in 5 anatomical sites while under ether anesthesia. After shaving the area, a single back wound approximately 1 cm square was made surgically into the skin overlying the scapula of each animal. The excisional wound extended through the epidermis, dermis, and panniculus carnosus muscle into the subcutaneous fatty connective tissue. Secondly, wedges were excised from the auricles of both ears of each rat. The wedges extended through the entire thickness of the ear and were approximately 3 mm at the base with their apex 3 mm from the edge of the ear. Finally, 2 palatal wounds were also made in each rat. On each side of the palate a surgical incision was made from a line 1 mm medial to the maxillary first molar, through epithelium and underlying connective tissue to the cemento-enamel junction of the tooth. (Fig 1 *A, B* and *C*).

In order to determine the location of granulation tissue and the peak times of DNA synthesis, four experimental animals were injured in the above manner at each of the following daily intervals before sacrifice: 10, 7, 5, 4, 3, 2, and 1. Twenty-eight experimental animals and 4 uninjured control animals were sacrificed at 10 AM on day 0. One hour before sacrifice, tritiated thymidine (0.5 μCi of ^{3}H-TdR/gm body weight; New England Nuclear, sp. act. 6.7 Ci/m mole) was injected intraperitoneally into each animal. Excised back and ear lesions were removed, fixed in Bouin's fixative, dehydrated, embedded in paraffin and step serial-sectioned at 6 μm. The wounded maxillas were embedded and sectioned similarly after decalcification in citrate-buffered formic acid. Slides containing sections through the wounds were dipped in NTB-2 Kodak nuclear emulsion and exposed in black bakelite boxes at 4°C for 2 weeks before development. The slides were then stained with hematoxylin and eosin (Fig 1 *D*).

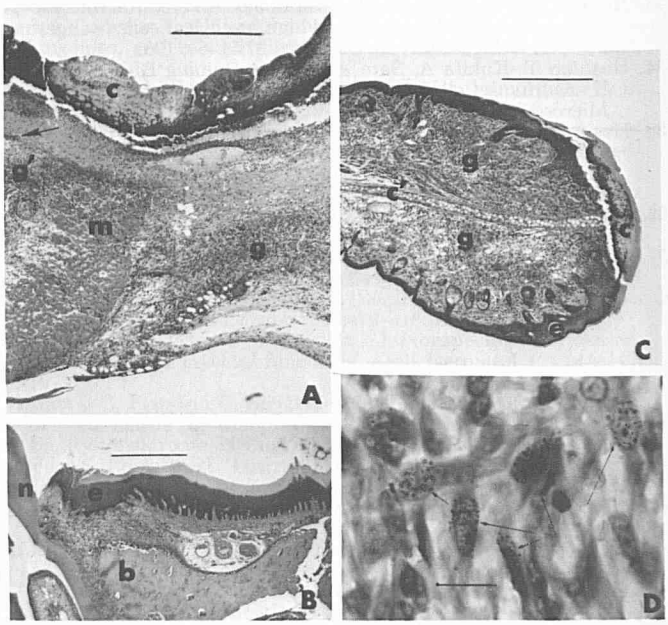

FIG 1. Three sites of injury (backs, maxilla, ear) at 72 hr following surgical excision. *Bars* in *A, B,* and *C* represent 0.5 mm. *A*, Back wound with granulation tissue (*g*) arising from subcutaneous tissue below interrupted panniculus carnosus muscle (*m*). Also, granulation tissue (*g'*) arising from panniculus adiposa lies beneath the leading edge of migrating epithelium (*arrow*). A fibrin clot (*c*) covers the wound. (reduced from × 38). *B*, Maxillary palatal mucosa from the surface of the first molar (*m*) to the palatal midline on the right, with epithelium (*e*) and connective tissue overlying alveolar bone (*b*) (reduced from × 32). *C*, One of 2 sides of ear wound. The fibrin clot (*c*) covers the cut surface of epithelium (*e*), connective tissue and cartilage (*c'*). Granulation tissue (*g*) was prominent around the striated muscle above the cartilage or in the fatty connective tissue below the cartilage (reduced from × 39). *D*, Autoradiograph of granulation tissue in wound base of back lesion. Five nuclei of fibroblast-like cells show heavy labeling (*arrows*) following tritiated thymidine injection (reduced from × 225). *Bar* represents 0.05 mm.

Manuscript received for publication August 8, 1977; accepted for publication February 22, 1978.

Supported by Dental Research Institute (NIDR Parent grant No. DE02731), and the School of Dentistry, University of Michigan.

Reprint requests to: Carl T. Hanks, 5224 School of Dentistry, University of Michigan, Ann Arbor, Michigan 48109.

Abbreviations:
DE: dermal extract
LE: liver extraction

Tissue Extract Preparation

Skins from approximately 100 neonatal rats were immersed in 55°C water for 30 sec and then immediately cooled on ice [11]. The epidermis and dermis were separated with forceps and minced in homogenizing buffer with surgical scissors. The dermis was homogenized in 0.02 M Tris-HCl buffer at pH 7.15 and centrifuged at 10,800 ×g for 30 min at 4°C. The supernate was then dialyzed against 0.02 M Tris-HCl buffer and the dialysate was lyophilized and stored at −20°C until reconstituted. The extract was reconstituted in 0.02 M Tris-HCl buffer, pH 7.15, to a protein concentrationof 10mg/ml as determined by the Lowry method [12]. Neonatal liver extract was prepared in a similar manner.

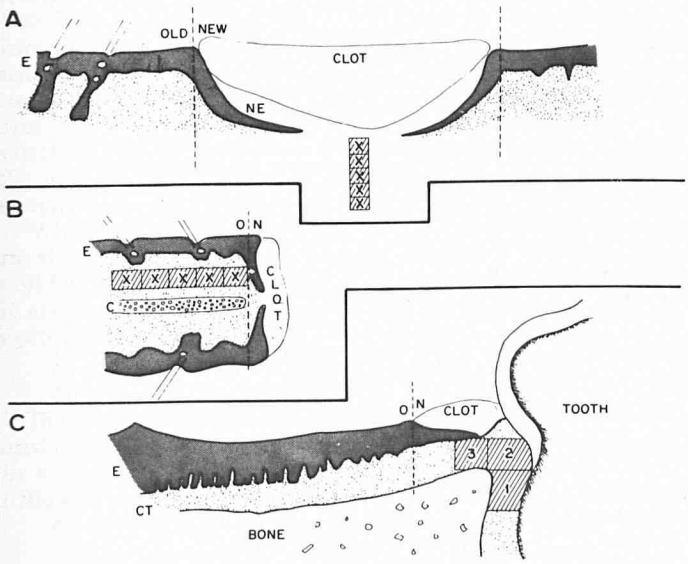

FIG 2. Wound sites in back (*A*), ear (*B*) and palate (*C*) diagrammed to show positions of fibrin clot, epithelium and connective tissue. The positions of the 0.0625 mm^2 fields ( ) in the wound bases, as well as relationship of new regenerating epithelium (*n*) and old residual epithelium (*o*) are also shown.

Effects of Tissue Extracts

After determining average times of peak DNA synthesis in each wound site, 48 experimental animals were each wounded in the same manner in the 5 anatomical sites. Four animals were injected subcutaneously in the neck region with 1 ml (10 mg protein/ml) dermal extract (DE) and 4 animals with a similar preparation of liver extraction (LE) at each of the following times before sacrifice: 24, 20, 16, 12, 8 and 4 hr. All 48 experimental animals and 4 surgical control animals, wounded similarly and injected with 1 ml saline 24 hr prior to sacrifice, were killed under ether anesthesia 72 hr after wounding. One hour before sacrifice, all animals were injected intraperitoneally with 0.5 μCi of ^{3}H-TdR/gm body weight. The tissues were processed as described previously.

ANALYSIS

The autoradiographs were viewed at a magnification of 400 × using a square net reticle. Each tissue field measured 0.0625 mm^2, i.e., 0.25 mm on the side. Only connective tissue nuclei labeled with more than 5 silver grains and not lining a capillary lumen were counted. Nuclear labeling per high power field (HPF) of connective tissue was determined for successive depths in each wound base (Fig 2*A*, *B* and *C*). In the back wounds, nuclear labeling/HPF was determined from a line just beneath the clot in the central portion of each wound at the successive depths of 0–0.25 mm; 0.25–0.5 mm; 0.5–0.75 mm; 0.75–1.0 mm and 1.0–1.25 mm. Thus, these fields included edematous connective tissue as well as granulation tissue. In ear wounds, nuclear labeling/HPF was determined as in the back wounds, from a line just beneath the clot, at successive depths of 0–0.25 mm; 0.25–0.5 mm; 0.5–0.75 mm; 0.75–1.0 mm and 1.0–1.25 mm in the wound base. Counts from connective tissue on both sides of the ear cartilage were included in this

TABLE I. *Nuclear labeling in wounded connective tissue following surgical excision*

Tissue Depth (mm)	Days Following Excision 1	2	3	4	5	7	10	C[a]
	Labeled Nuclei/HPF ($\bar{X}$ ± SEM)							
Table IA. Back tissue								
0–0.25	0	0.33 ± 0.3	0	3.5 ± 3.5	25.6 ± 3.9	14.7 ± 1.8	6.5 ± 3.7	4.3 ± 1.9
0.25–0.50	0.5 ± 0.5	0.67 ± 0.3	1.8 ± 1.2	44.5 ± 4.1	24.6 ± 6.6	7.0 ± 1.2	12.0 ± 1.6	0
0.5–0.75	0.3 ± 0.3	2.0 ± 2.0	30.8 ± 9.1	42.0 ± 8.2	20.0 ± 5.7	7.8 ± 2.2	9.2 ± 1.7	1.0 ± 0.1
0.75–1.0	1.3 ± 0.9	0	25.3 ± 5.5	21.5 ± 4.3	14.3 ± 3.4	3.3 ± 1.1	4.3 ± 1.5	0
1.0–1.25	3.3 ± 3.3	0	2.3 ± 1.9	15 ± 0.0	5.3 ± 2.7	4.0 ± 1.0	3.0 ± 0.9	0
1.25–1.50	0	—[c]	0	9 ± 1.7	1	2.5 ± 1.7	2.5 ± 1.9	0
GT[b]	12.8 ± 5.2	17.3 ± 0.6	42.1 ± 6.6	35.3 ± 2.4	23.3 ± 3.0	—	—	
Table IB. Ear tissue								
0–0.25	0.1 ± 0.1	10.8 ± 1.8	24.5 ± 2.7	34.3 ± 5.2	38.9 ± 5.8	27.3 ± 2.3	8.7 ± 1.2	0.7 ± 0.2
0.25–0.50	2.1 ± 0.6	17.3 ± 2.1	21.0 ± 2.6	30.2 ± 5.7	21.0 ± 5.7	12.1 ± 2.1	4.5 ± 1.1	0
0.50–0.75	1.4 ± 0.6	13.2 ± 1.5	10.9 ± 1.6	13.2 ± 3.4	7.9 ± 2.6	4.8 ± 0.9	1.4 ± 0.3	0
0.75–1.00	2.1 ± 0.5	11.4 ± 1.2	6.4 ± 1.0	9.5 ± 2.3	2.3 ± 0.8	3.7 ± 0.9	1.2 ± 0.3	0
1.00–1.25	1.7 ± 0.5	12.9 ± 1.3	5.7 ± 0.8	5.9 ± 0.5	2.9 ± 0.6	2.0 ± 0.5	0.8 ± 0.2	0
Table IC. Palate tissue								
Zones[d]								
1.	8.0 ± 2.9	19.3 ± 3.6	11.1 ± 2.4	10.6 ± 2.1	9.1 ± 3.0	7.5 ± 2.5	6.8 ± 1.8	6.9 ± 1.2
2.	7.2 ± 1.9	11.3 ± 2.9	21.6 ± 5.0	18.1 ± 2.9	10.1 ± 1.8	4.0 ± 1.6	4.0 ± 1.5	2.0 ± 1.4
3.	11.2 ± 2.2	17.3 ± 3.2	26.8 ± 4.2	23.5 ± 4.0	21.8 ± 8.9	6.6 ± 2.1	4.3 ± 1.1	2.1 ± 0.6

[a] Unoperated control animals.
[b] Granulation tissue: Counts taken from most densely labeled zones, regardless of depth.
[c] — Samples lost during processing.
[d] Connective tissue zones in the palatal wound base as described in Methods under "Analysis."

TABLE II. *Effects of DE on wounded connective tissue*

Tissue Depth (mm)	Injection Time Before Sacrifice (hr)						
	4	8	12	16	20	24	SC[a]
	Labeled Nuclei/HPF ($\bar{X}$ ± S.E.M.)						
	Table IIA. Back tissue						
0–0.25	5.7 ± 2.9	0	5.6 ± 3.5	0.1 ± 0.1	0	1.5 ± 1.2	—[b]
0.25–0.50	27.8 ± 8.3	33.8 ± 3.7	43.8 ± 5.1	5.4 ± 2.5 (−87%)[c]	8.1 ± 4.9 (−81%)	23.3 ± 3.0 (−45%)	42.3 ± 3.2
0.50–0.75	8.8 ± 5.8	8.2 ± 1.6	13.1 ± 2.7	12.3 ± 3.8	5.4 ± 2.1	6.3 ± 2.1	16.7 ± 5.9
0.75–1.00	6.0 ± 2.0	7.6 ± 3.1	8.7 ± 2.8	8.3 ± 2.0	3.5 ± 1.9	3.3 ± 1.7	21.0 ± 10.0
1.00–1.25	0	4.0 ± 0.6	4.3 ± 2.4	3.0 ± 0.1	5.0 ± 2.0	8.0 ± 0.1	—
GT[d]	32.3 ± 4.1	38.4 ± 2.4	35.7 ± 6.6	15.2 ± 4.6*[e] (−58%)	15.9 ± 5.8***[e] (−57%)	23.6 ± 4.0 (−36%)	36.6 ± 2.4
	Table IIB. Ear tissue						
0–0.25	32.8 ± 4.0	27.4 ± 2.4	27.2 ± 2.9	20.8 ± 2.0*[f] (−24%)	19.4 ± 2.3*[f] (−29%)	23.8 ± 2.9	27.5 ± 1.5
0.25–0.50	17.4 ± 5.0	14.7 ± 2.8	17.1 ± 2.6	11.9 ± 1.9	10.9 ± 2.3	19.5 ± 3.4	15.4 ± 3.3
0.50–0.75	13.3 ± 3.7	10.0 ± 1.4	9.0 ± 1.5	7.0 ± 1.8	4.5 ± 1.3	9.4 ± 1.8	10.8 ± 2.1
0.75–1.00	8.3 ± 1.2	7.5 ± 1.4	9.5 ± 1.7	4.7 ± 1.0	4.3 ± 1.9	7.6 ± 1.4	6.9 ± 1.5
1.00–1.25	9.1 ± 1.7	6.1 ± 1.3	7.7 ± 1.3	5.9 ± 1.4	2.3 ± 0.5	3.7 ± 0.8	4.3 ± 0.7
	Table IIC. Palate tissue						
Zones[g]							
1	26.5 ± 6.4	16.4 ± 3.9	16.7 ± 2.9	9.2 ± 1.6**[h] (−59%)	11.4 ± 5.1 (−49%)	13.1 ± 3.1 (−42%)	23.1 ± 2.8
2	22.2 ± 5.6	21.2 ± 3.6	10.2 ± 1.9*[h] (−58%)	11.9 ± 4.2*[h] (−51%)	11.5 ± 3.4*[h] (−53%)	13.5 ± 3.0 (−45%)	24.5 ± 2.6
3	33.3 ± 3.3	30.3 ± 1.9	33.5 ± 5.9	14.7 ± 1.9*[h] (−68%)	24.9 ± 3.9***[h] (−45%)	33.3 ± 5.4	45.5 ± 2.7

[a] Surgical control animals. Operated, and injected with 1 ml physiologic saline 24 hr before sacrifice.
[b] Tissue samples lost during processing.
[c] Percent reduction of nuclear labeling/HPF below surgical control values.
[d] Granulation tissue. Counts taken from most densely labeled zones, regardless of depth.
[e] Student *t*-test of significance: experimental vs control autoradiographs. * $0.02 > P > 0.01$ and ** $0.05 > P > 0.02$; all other granulation tissue values, $P > 0.05$.
[f] Student *t*-test of significance: experimental vs control autoradiographs. * $0.05 > P > 0.02$; all other values, $P > 0.05$.
[g] Connective tissue zones in palatal wound base, as described in Methods under "Analysis."
[h] Student *t*-test of significance: experimental vs control autoradiographs. * $P < 0.001$; ** $0.01 > P > 0.001$; and *** $0.02 > P > 0.01$; all other values, $P > 0.05$.

data. In the maxillary mucosal wounds, 0.0625 mm^2 zones of connective tissue were counted as shown in Fig 2*C*. Zone 1 lay between the molar tooth surface and the alveolar bone crest at the level of the crest. Zone was superficial to zone 1. Zone 3 was superficial to the crest of alveolar bone and adjacent to zone 2. The average($\bar{X}$ ± SEM) for multiple sections from each of our animals was determined for each level or zone of connective tissue in each wound site.

Nuclear labeling of epithelium was expressed as basal cell DNA synthesis index per 0.25 mm linear surface distance of epithelium. In the back and ear wound margins only interfollicular epithelium was counted. All epithelial basal cells were counted in palatal wound margins, since the epithelium is not interrupted by adnexa. The epithelium in all cases was separated into new regenerating zones and old residual zones, as shown in Fig 2*A, B,* and *C*.

The Student *t*-test was used to determine the confidence levels for differences between experimental and control animals.

RESULTS

The levels of nuclear labeling/HPF of connective tissue in wound bases of back, ears and palates are given in Tables I*A*, I*B*, and I*C*, respectively. For the back lesions, the areas of greatest connective tissue proliferation and nuclear labeling were also counted and specially designated as granulation tissue. Granulation tissue and increased nuclear labeling were apparent at 3 days after injury at 0.5–1.0 mm below the fibrin clot in the back wounds, at 0–0.5 mm from the clot in the ear wounds, and in all 3 zones in the palatal wounds.

Nuclear labeling of connective tissue in the same sites following DE injection are shown in Tables IIA, IIB and IIC. The most prominent reductions (57–87%) of nuclear labeling were observed in the back lesions, in granulation tissue which occurred at the 0.25–0.5 mm level, 16 and 20 hr following the injection of extract (Table IIA). Over a 50% reduction was also observed in all 3 zones of the palatal lesions (Table IIC). The least effect of DE extract was noted in the granulation tissue (0–0.25 mm) of the ear wounds (Table IIB). On the other hand, LE injection under the same conditions did not cause any significant reduction (i.e., $P < 0.05$) of nuclear labeling in any of the sites.

The DNA synthesis indices of epithelial cells in the wound margins were measured particularly to determine whether the reduction of nuclear labeling at 16–20 hr after injection of DE also occurred in epithelial basal cells. The figures in Fig 3*A*, *B* and C shows the mean DNA synthesis indices for groups of animals injected with DE at each of the specified time intervals. In the back wound margin (Fig 3*A*), zone 2 of the new epithelium lay under the clot and averaged 2.24 fields (0.56 mm) in length. Zone 1 of the new epithelium lay on either side of the clot and averaged 1.3 fields (0.33 mm) in length. Neither of these fields contained hair follicles. The residual epithelial zones

each contained an average of 33.25 basal cells/0.25 mm surface distance of interfollicular epithelium. In the ear wound margins (Fig 3*B*), the new nonfollicular epithelium was only about 0.25 mm long (zone 1). The other zones of old residual epithelium contained 52.46 basal cells/0.25 mm surface distance of interfollicular epithelium. Fig 3*C* shows that, unlike back and ear epithelium, the number of basal cells per 0.25 mm surface distance of uninjured nonfollicular palatal epithelium varied from around 70/field near the first molar (new zone 2) to over 100/field near the midline (residual zone 6). This was due to increased papillation of the epithelium as it approaches the midline. The DNA synthesis index of the unoperated animals, however, was the same in all zones. In contrast, the DNA synthesis indices of all groups of experimental animals injected with DE increased in residual marginal epithelium (zones 1 and 2), in a manner similar to marginal epithelium of uninjected surgically injured animals (Fig 4).

When Fig 3*A*, *B* and *C* are compared, it can be seen that at no time intervals is there consistent lowering of epithelial basal cell DNA synthesis activity in all 3, or even 2 of 3 tissues. Thus, it appears that dermal extract did not have a parallel effect on epithelium as on connective tissue.

DISCUSSION

In the present study, the dermal extract appeared to consistently inhibit DNA synthesis of proliferating connective tissue in all animals. This inhibition was seen generally at 16–20 hr following injection. This could be accounted for by the interruption of the cell cycle of proliferating fibroblasts by a factor in the tissue extract, rather than generalyzed cytotoxicity. Such a tissue-specific "negative feedback" mechanism operating via the blood stream has been described for other tissues such as rat kidney [13] and rat liver [14,15].

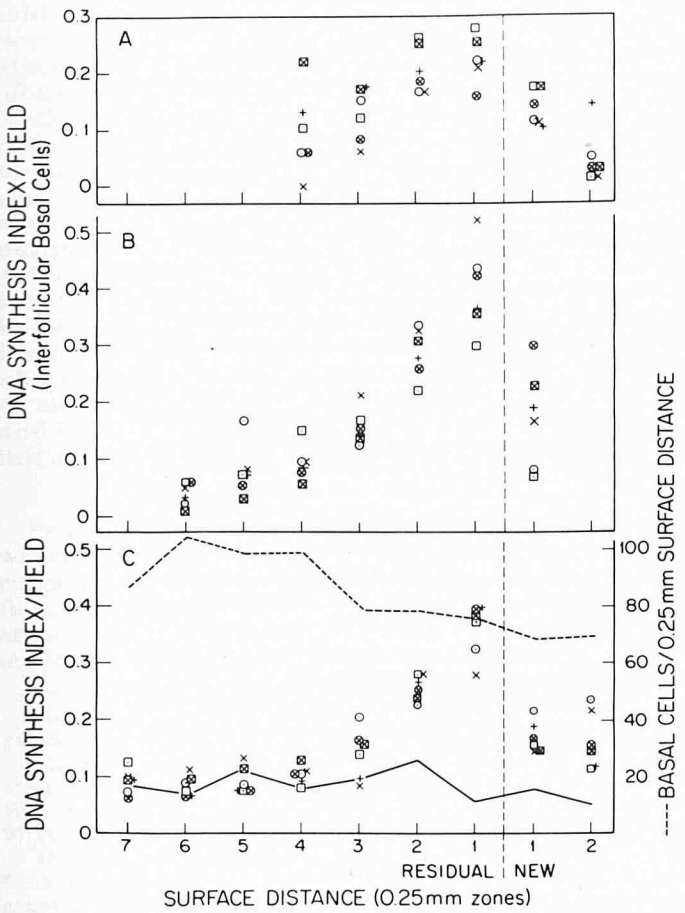

FIG 3. DNA synthesis indices from epithelium in wound margins of back (*A*), ear (*B*) and palatal (*C*) lesions. The symbols for each new and residual zone represent the 6 time intervals (before sacrifice time) at which dermal extract was injected into the experimental animals: +, 24 hr; , 20 hr; ,16 hr; ×, 12 hr; , 8 hr; , 4 hr. In graph *C*, both DNA synthesis indices (——) and number of basal cells per zone (----) in uninjured palatal epithelium are also shown.

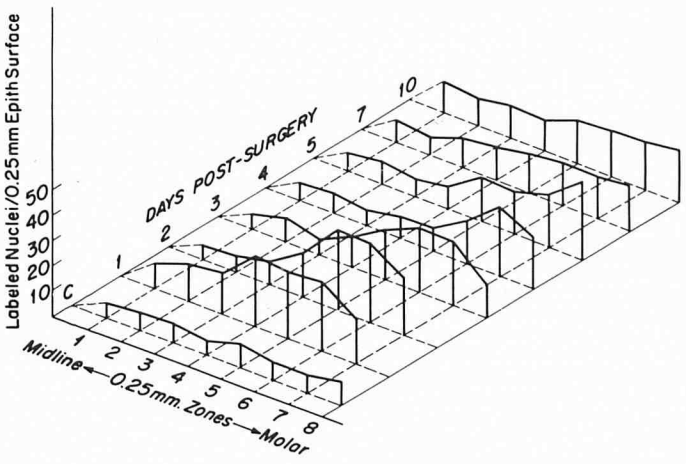

FIG 4. Labeled epithelial nuclei per 0.25 mm linear surface distance from wound margins of healing palatal lesions from day 1 through day 10. None of these animals were injected with dermal or liver extracts. In the majority of animals, the zone immediately adjacent to the first molar was incompletely epithelialized until the third day after surgery. All zones show increased labeling over uninjured control (*c*) animals.

The timing of the inhibition of DNA synthesis appeared to be discrete, since in most cases extracts injected at 20 and 16 hr before sacrifice depressed DNA synthesis, while extracts injected closer to the time of sacrifice did not. It is not apparent at which point in the cell cycle the extract was active, since we did not know the time required for the factor to reach an adequate concentration in the wound sites or the length of the tissue fibroblast cell cycle. Furthermore, it is possible that "resting" fibroblasts were blocked from entering G_1 form the G_0 state [16] rather than being inhibited after they were already stimulated. Studies with other experimental models have shown that early events following stimulation to proliferate, perhaps at a "restriction point" in G_1 [17] are important for later DNA synthesis and mitosis. Inhibition of protein synthesis during the first 8 hr following isoproterenol stimulation resulted in depressed DNA synthesis 20 hr later in mouse salivary gland [18]. Further, inhibition of RNA synthesis in the first 4 hr after hepatectomy resulted in depressed DNA synthesis 12–18 hr later [19]. Finally, injected epidermal "G_1" chalone depressed DNA synthesis in mouse epidermis *in vivo* maximally between 9–20 hr after injection [20,21].

Earlier studies and conceptualizations described epidermal chalones as tissue specific, but species nonspecific, factors which could act locally through a diffusion gradient or more generally through the circulation [22,23]. In this study, we used a large amount of tissue extract (10 mg per animal) to determine if it were capable of suppressing DNA synthesis, at an optimum time point for observation. Under physiologic conditions, this factor, which may be present in smaller quantities in mature connective tissue cells and may spread by gradient diffusion through the intercellular milieu, may help to control growth in embryonic, wounded or even neoplastic connective tissue. In a wound site, serum factors and the reduced concentration of fibroblastic chalone could conceivably allow proliferation of granulation tissue until a critical number of fibroblasts and concentration of chalone were again present in the intercellular milieu.

REFERENCES

1. Grillo HC: Deviation of fibroblasts in the healing wound. Arch Surg 88:218–224, 1964

2. Gillman T: On some aspects of collagen formation in localized repair and in diffuse fibrotic reactions to injury, Treatise on Collagen, Part B. Edited by BS Gould. New York, Academic Press, 1968, pp 331–407
3. Ordman LJ, Gillman T: Studies in the healing of cutaneous wounds. Arch Surg 93:857–882, 1966
4. Kunkel TA, Meyer RR, Alexander JW: Isolation and purification of mediators of cell proliferation. Arch Surg 110:884–887, 1975
5. Kohler N, Lipton A: Platelets as a source of fibroblast growth-promoting activity. Exp Cell Res 87:297–301, 1974
6. Ross R, Glomset J, Kariya B, Harker L: A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. Proc Natl Acad Sci USA71:1207–1210, 1974
7. Houck JC, Sharma VK, Cheng RF: Fibroblast chalone and serum mitogen (anti-chalone). Nature New Biol 246:111–113, 1973
8. Houch JC: Fibroblast chalone and a putative circulating anti-chalone, Chalones. Edited by JC Houck. New York, American Elsevier Publ Co, Inc, 1976, pp 247–264
9. Weiss P, Kavanau JL: A model of growth and growth control in mathematical terms. J Gen Physiol 41:1–47, 1957
10. Houck JC, Weil RL, Sharma VK: Evidence for a fibroblast chalone. Nature New Biol 240:210–211, 1972
11. Marrs JM, Voorhees JJ: A method for bioassay of an epidermal chalone-like inhibitor. J Invest Dermatol 56:174–181, 1971
12. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275, 1951
13. Saetren H: A principle of auto-regulation of growth. Production of organ-specific mitose-inhibitors in kidney and liver. Exp Cell Res 11:229–232, 1956
14. Glinos AD, Gey GO: Humoral factors involved in the induction of liver regeneration in the rat. Proc Soc Exp Biol Med 80:421–425, 1952
15. Stich HFJ, Florian ML: The presence of a mitosis inhibitor in the serum and liver of adult rats. Canad J Biochem 36:855–859, 1958
16. Baserga R: Multiplication and Division in Mammalian Cells, New York, Marcel Dekker, Inc, 1976, pp 175–188
17. Pardee AB, de Asuá LJ, Rosengurt E: Functional membrane changes and cell growth: Significance and mechanism, Control of Proliferation in Animal Cells. Edited by B Clarkson, R Baserga. Cold Spring Harbor Laboratory, 1974, pp 547–561
18. Sasaki T, Litwack G, Baserga R: Protein synthesis in the early prereplicative phase of isoproterenol-stimulated synthesis of deoxyribonucleic acid. J Biol Chem 244:4831–4837, 1969
19. Fujioka M, Koga M, Lieberman I: Metabolism of ribonucleic acid after partial hepatectomy. J Biol Chem 238:3401–3406, 1963
20. Hennings H, Elgjo K, Iverson OH: Delayed inhibition of epidermal DNA synthesis after injection of an aqueous skin extract (chalone). Virch Arch B 4:45–53, 1969
21. Marks F: A tissue-specific factor inhibiting DNA synthesis in mouse epidermis, Chalones: Concepts and Current Researches Washington, D.C., National Cancer Institute Monograph No. 38, 1973, pp 79–92
22. Iverson OH: A homeostatic mechanism regulating the cell number in epidermis: Its relation to experimental skin carcinogenesis, Proc First Int Congr Cybernetic Med. Edited by A Masturo. Naples, Societa Internazionale di Medicina Cibernetic, 1960, pp 420–430
23. Bullough WS, Laurence EB: The control of epidermal mitotic activity in the mouse. Proc Roy Soc B 151:517–536, 1960

Announcement

The Society for Investigative Dermatology is pleased to announce that it will again offer Research Fellowship Awards. These are designed for individuals embarking on academic careers who wish to spend one or more years working primarily in basic research. This training must be obtained under the guidance of a sponsoring investigator. The Fellowships are not intended to support individuals who have substantial teaching and/or patient care commitments. The award will provide a stipend of $16,000.00 per year. Applications may be obtained from: W. Mitchell Sams, Jr., M.D., Secretary-Treasurer, The Society for Investigative Dermatology, Department of Dermatology, North Carolina Memorial Hospital, Chapel Hill, North Carolina 27514. Deadline for submitting applications is October 1, 1978. Awards will be announced by December 15, 1978 and may commence any time between January 1 and July 1, 1979.